Nucleic Acid · Structure

Calculated T_m (°C, average base composition)

n=	8	7	6	5
	38	33	25	15
	33	25	15	3
	25	15	3	-14
	51	46	40	31
	46	40	31	21
	40	31	21	11

FIGURE 1

Biotin (•)

Streptavidin
Surface

(N)
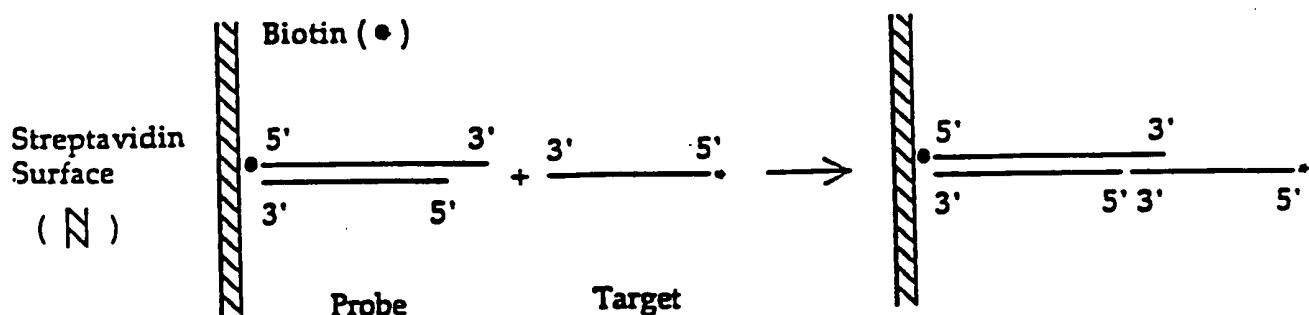
A. Basic scheme for positional SBH
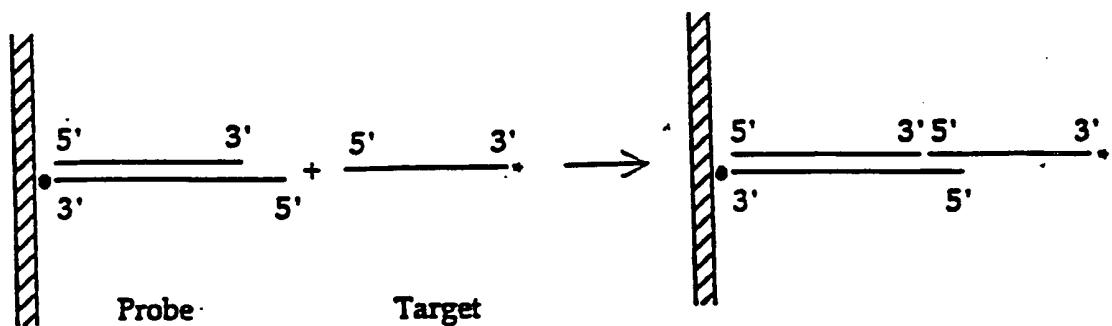
Probe   Target

B. Alternate scheme

FIGURE 2

Ligation of target DNA with probe

A.

B.

FIGURE 3

5'GCGGCCGC — GACGC —— 3'
3'CGCCGGCG — CTGCG —— NNNNN 5'

Chemical synthesis

→ Polymerase

5' GCGGCCGC — GACGC —— NNNNN 3'
3' CGCCGGCG — CTGCG —— NNNNN 5'

↓ Blunt end ligate clone, pick colonies

↓ Not I cleavage

5'   GGCCGC — GACGC —— NNNNN ————
3'   CG — CTGCG —— NNNNN ————

↓ Fill in with ppp G and biotinylated ppp C●

5'   GGCCGC — GACGC —— NNNNN ————
3'   CCGGCG — CTGCG —— NNNNN ————

5'   GGCCGC — GACGC —— 3'
3'   CCGGCG — CTGCG —— NNNNN 5'
●●

←

Cleavage with Hga I
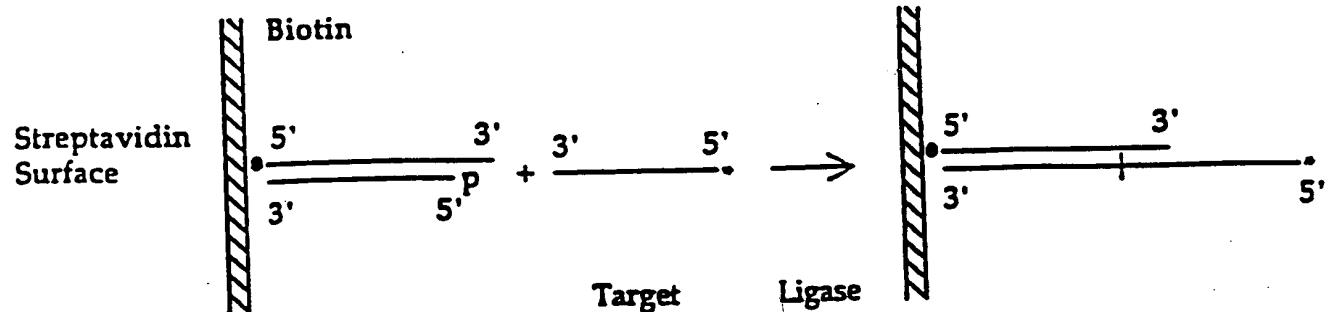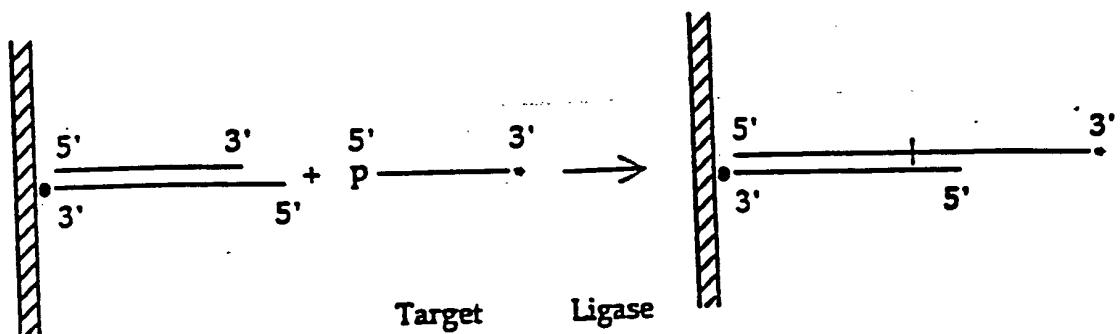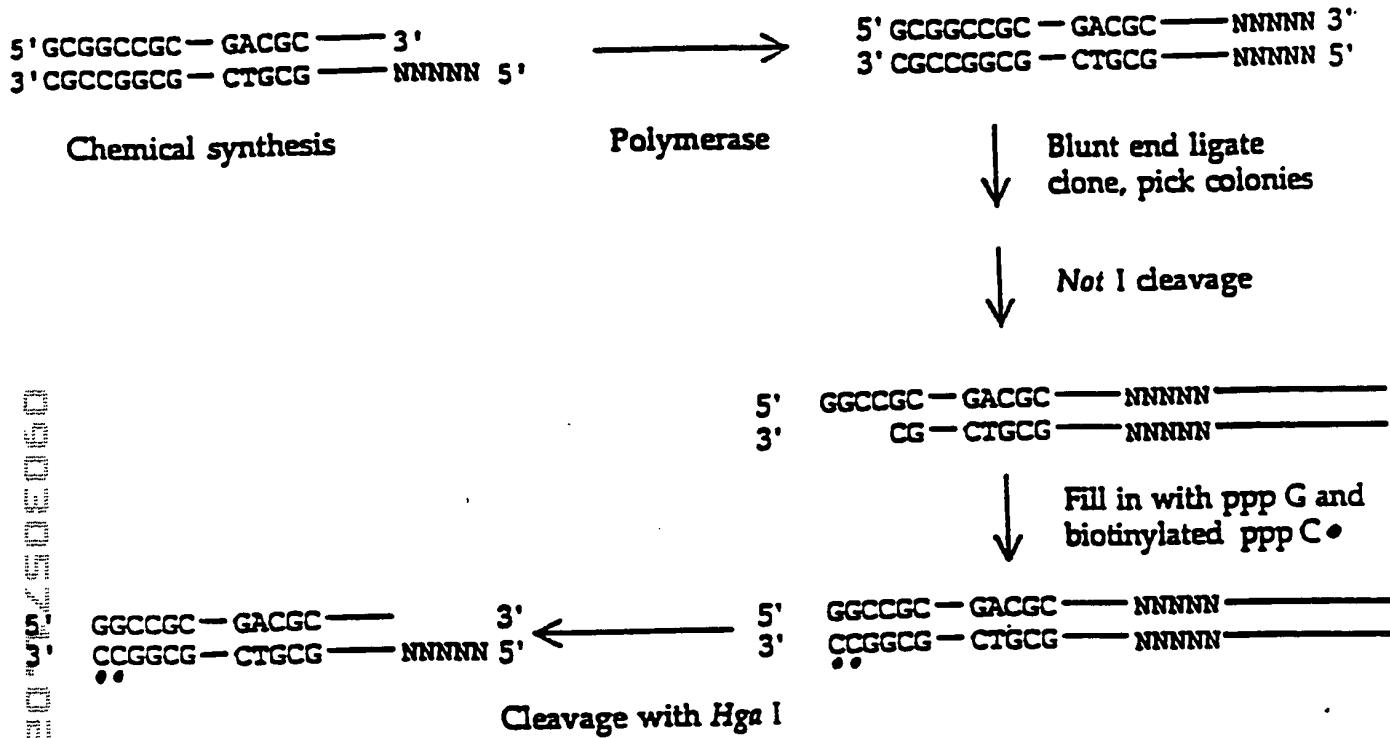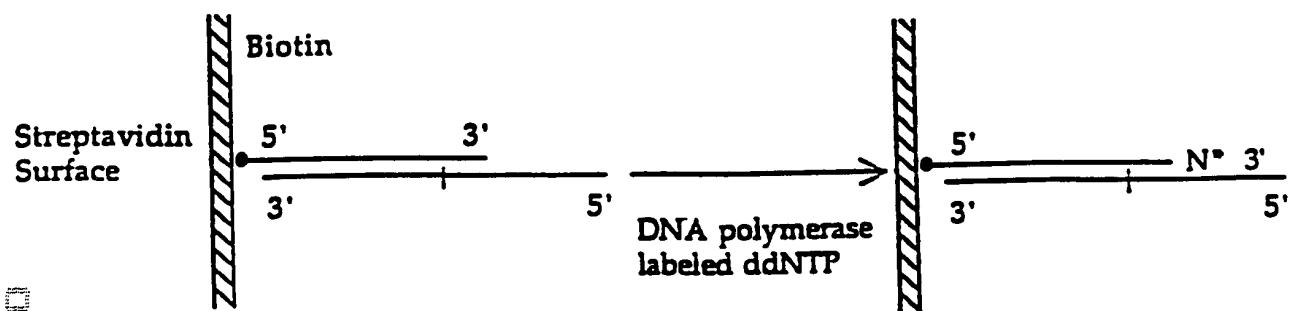
FIGURE 4

Reading an extra target base by 3' extension of the probe

**FIGURE 5**
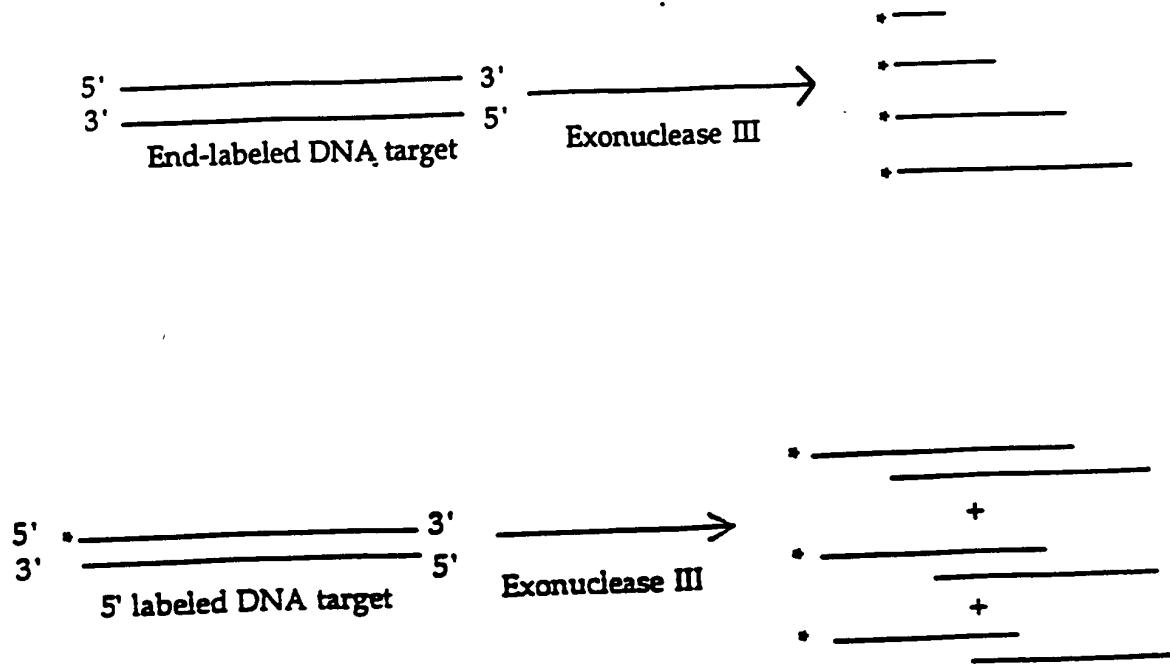
Preparation of a nested set of DNAs
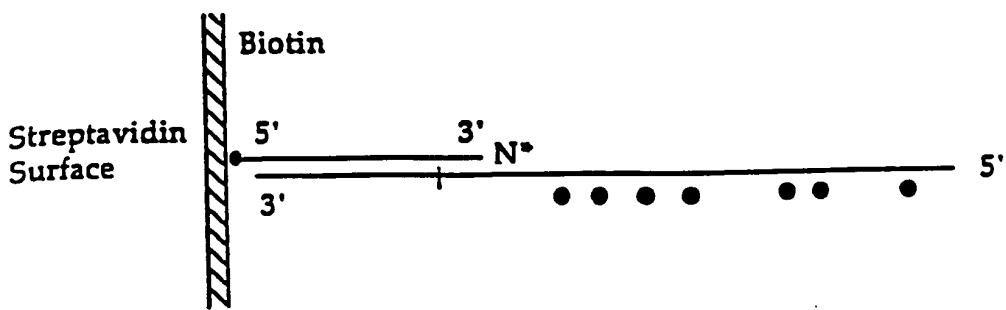
FIGURE 6

Positional information from ratio of internal label (●) to extension label (*). A 5' label could also be used.

FIGURE 7

Reading an extra target base

5'————————— AAGCTA ————————— 3'
                 TCGAT ————————— • 5'

↓ pppTp

5'————————— AAGCTA ————————— 3'
              pTTCGAT ————————— • 5'

↓

5'————————— AAGCTA ————————— 3'
                TTCGAT ————————— • 5'
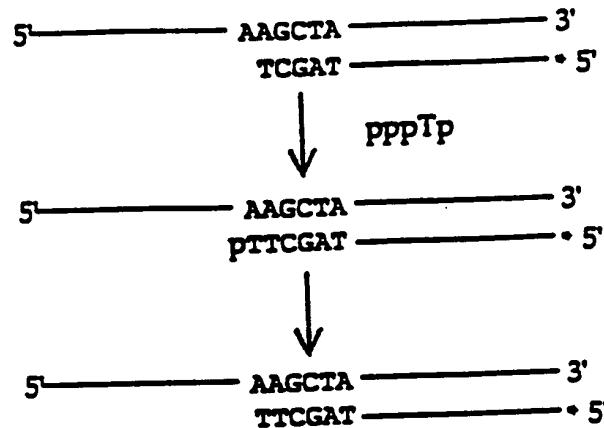
Extension will fail with pppAp, pppGp, and pppCp

A. 3' extension of the target with a pppNp prior to phosphatase treatment

Streptavidin Surface | Biotin

5'————NAGCTA 3' + 3'TTCGAT ————— • 5' → 5'———— AAGCTA 3'
3'   5'   3'   TTCGAT ————— • 5'
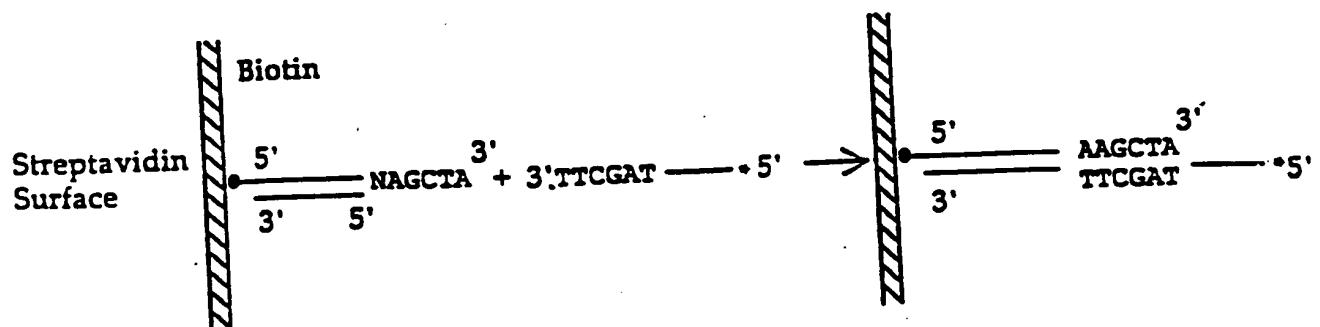
Array with one degenerate base

B. Hybridization and ligation

FIGURE 8

LABEL INTENSITY

Chain Terminator:	T	G	A
SEQUENCE			
GGAAT	2A, 2G	—	2G
AAGGT	2A, 2G	2A	—
GAAGT	2A, 2G	—	1G
AGGAT	2A, 2G	1A	—
AGAGT	2A, 2G	1A	—
GAGAT	2A, 2G	—	1G
GGGAT	1A, 3G	—	3G
GGAGT	1A, 3G	—	2G
GAGGT	1A, 3G	—	1G
AGGGT	1A, 3G	1A	—
AAAGT	3A, 1G	3A	—
AAGAT	3A, 1G	2A	—
AGAAT	3A, 1G	1A	—
GAAAT	3A, 1G	—	1G
GGGGT	4G	—	1T, 4G*
AAAAT	4A	1T, 4A*	—

Four color analysis of sequence extension of the 3' end of
the probe. Label intensity shown does not include contribution
from the 3' terminator.

* Plus additional possible residues

FIGURE 9
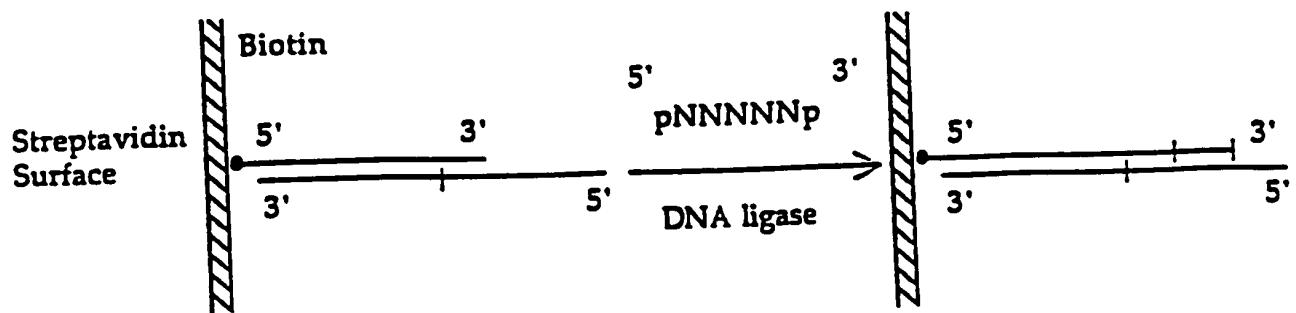
Extension of the probe by ligation of a blocked pentanucleotide.
The pentanucleotide is 3'-blocked to prevent polymerization.

FIGURE 10
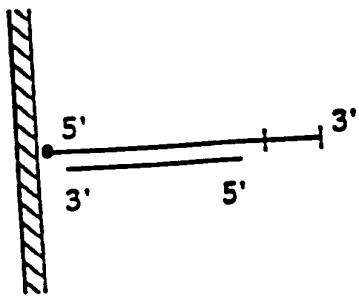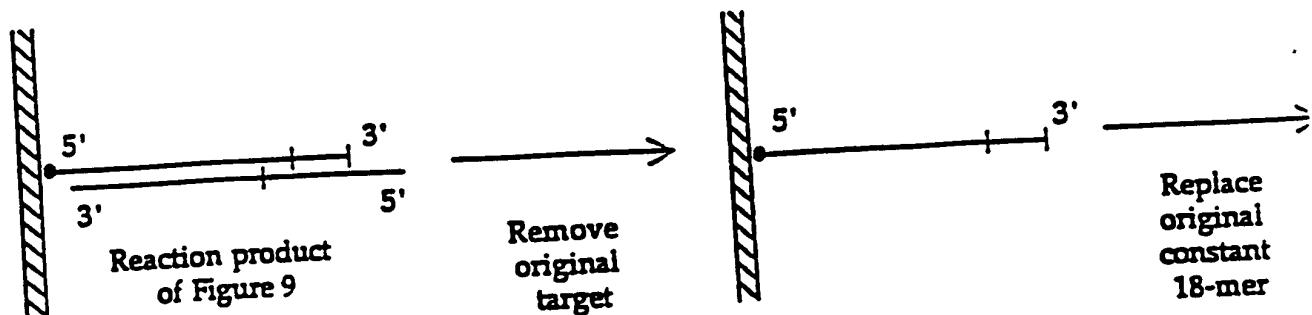
Preparation of a customized probe containing a 10 bp sequence
present in the original target DNA

FIGURE 11

FIGURE 12

FIGURE 13

Master
array

Incubate with biotinylated
complementary strand

Synthesis of
complementary array

Contact above T_m

Streptavidin-coated filter or
plate array compressed by
offsetting

Incubate with
complementary strands

Full, finished array

stv
|
b

stv
|
b
‖ ‖
b
3' 5'

pol I
————————→
4 dNTP's

stv
|
b
‖ ‖
b
3' 5'

3'
‖
b
|
stv

5' 3'
‖ ‖
b
|
stv

FIGURE 14